Supplemental Materials

Production and characterization of N15TCRαβ and N15TCRαβΔFG T cell lines and IL-2 assay. 58 $\alpha^-\beta^-$ cells previously transfected to express CD8αβ and N15TCRαβwt or the N15TCRαβΔFG mutant (1) were sorted to equivalently match both TCR and CD8 expression levels on the two cell lines. Then R8 (ATCC) cells as APCs were loaded with VSV8 peptide concentrations ranging from 0.1 to 100 μg/ml to stimulate N15 TCRαβwt and N15αβΔFG expressing cells. Responding cells were added in triplicate at a density of $2x10^5$ cells per well in 96 well plates using complete DMEM media supplemented with 1ng/ml PMA (Sigma). Cells in replicate wells were stimulated with 10ng/ml of PMA and 500ng/ml of Ionomycin (Sigma) as a positive control. After 18 h of incubation, plates were centrifuged and 50μl was removed for IL-2 analysis. Conditioned media was analyzed using mouse IL-2 bead assay from (BD biosciences) according to manufacturer's protocol on a FACSAria flow cytometer. Anti-CD3 mAb 2C11 and anti-CD8α were used for to quantitate surface TCR complexes and CD8αα and CD8αβ heterodimers (2).

Antibodies (Abs) and flow cytometric analysis. The following mAbs were used: FITC-conjugated anti-Vβ 5.2 (MR9.4) (ebioscience), PE-conjugated anti-TCR Cβ (H57-597) (BD Pharmingen), PE-conjugated anti-CD3ε (145-2C11) (ebioscience), APC-conjugated anti-CD8α (CD8a) (Biolegend), FITC-conjugated anti-CD8β (CD8b.2) (Biolegend) and PE-conjugated anti-H-$2K^b$ antibody (AF6-88.5) (ebioscience). For flow cytometry, single-cell suspensions of wt or ΔFG cells were prepared at 3×10^6 cells/ml in PBS containing 2% FCS. Those cells were single-color stained with the Abs at saturating concentrations. After incubation on ice for 20 minutes, the stained cells were transferred to a falcon tube and characterized by flow cytometry. FACScan (BD Biosciences) and FlowJo software (Tree Star) were used for all samples. Dead cells were excluded from the analysis by forward and side scatter gating. For assessing TCR expression further on both N15αβwt and N15αβΔFG cells, saturating amounts of FITC-conjugated anti-Vβ

5.2 were employed. After 20-minute incubation on ice, the stained cells were subjected to flow cytometry analysis.

Partial reduction of anti-biotin antibody. Anti-biotin antibodies (Sigma-Aldrich) at 10mg/ml were dissolved in the reaction buffer (pH=7.4) containing 20mM sodium phosphate (Sigma-Aldrich), 0.15 M NaCl (Sigma-Aldrich) and 5mM EDTA (Sigma-Aldrich). A 500-fold molar excess of 2-MEA (Sigma-Aldrich) over the concentration of anti-biotin antibody was added in the solution, followed by 90 minutes incubation at 37°C. The reaction product was purified using a 30 MBS desalting column (Millipore). Purified product was immediately used for the SMCC reaction.

Covalently coupled half anti-biotin antibody to DNA. A 3500 base-pair DNA linker was constructed by PCR using a M13mp18 plasmid as template with one forward oligonucleotide primer containing a Dig tag at the 5' end (5'-Dig-AAT CCG CTT TGC TTC TGA CT-3') and one reverse oligonucleotide primer containing an amino tag at the 5' end (5'-NH_2-TTG AAA TAC CGA CCG TGT GA-3'). Following PCR, the TE buffer in the PCR product was changed to PBS buffer using a 6 MBS desalting column (Millipore). A volume of 30 µl NH_2-DNA-Dig solution (conc. ~120 ng/µl) was incubated with 30 µl 10 mg/ml freshly made Sulfo-SMCC dissolved in dd-water at 4°C for 2 hours. A second buffer changing procedure was performed for the DNA-SMCC mixture by changing the PBS buffer to reaction buffer used for partial reduction of anti-biotin antibody. Maleimide-DNA-Dig was incubated with the purified half anti-biotin antibody for 12 hours at 4^oC in order to covalently couple the free thiol group of half anti-biotin antibody to the maleimide group on the DNA. The functionalized DNA can be kept in reaction buffer at -20^oC for 2 months without degradation of function.

H28 measurements

Bond lifetime vs force plots show that, in the presence of H28, bond lifetime increases with force up to 15 pN (Catch bond) followed by decrease of lifetime with force for Vsv8 (red) and

L4 (purple). Bond lifetime decreases with force for sev9 (blue). H28 measurement results and fits are found in Figure S1.

Catch bond fits. To parameterize and characterize the general shape of each catch bond curve, we fit the force vs bond lifetime data to a standard model for catch/slip bonds that includes two bound states each of which can unbind, where the force dependence of each state is given by Bell's model (3) and dissociation is either by a catch or slip pathway:

$$\frac{1}{t_{on}} = k_c^0 . e^{f.xc/kT} + k_s^0 . e^{f.xs/kT}.$$

Subscripts c and s indicate the catch and slip pathways, respectively, and t_{on} is the average bond lifetime, where k^0_c and k^0_s are the unloaded off rates and x_c and x_s are distance to transition state for catch and slip bonds respectively (4, 5). The fit results are detailed in Table S1. Residual plots for Figure 2 and Figure S1 catch bond fits and are found in Figure S2.

Analysis of TCR and Ig pdb files

High resolution X-ray structures of TCR and Ig (Fab) were selected from the structure deposition website, PDB, for statistical analysis. To calculate the buried surface area (BSA) of the two Ig domains of each TCR or Ig (Fab) chain, each chain was isolated and saved as a new pdb file (3-D structure coordinates file) respectively, using gedit (an edit tool). This tool was further used to delete the short linker between the constant and variable Ig domains (C domain and V domain). From the selected TCR and Ig structures (PDB code and resolution are listed in Tables S2 and S3), we obtained numbers for TCR_CαVα, TCR_CβVβ, TCR_CβVβΔFG, Fab_ChVh, Fab_CκVκ, Fab_CλVλ pdb files without the linker between the C and V domains. The individual BSAs of TCR_CαVα, TCR_CβVβ, TCR_CβVβΔFG (Table S2) or Fab_ChVh, Fab_CκVκ, Fab_CλVλ (Table S3) were calculated by importing the corresponding pdb file into the PISA program (Proteins, Interfaces, Structures and Assemblies Program). To easily see the difference in BSA between the two chains of the same structure, the ratio was calculated (Table S2 and S3) and the mean values of the ratios (Table S4) are depicted in Figure S2. Table S4 summarizes the

statistical data in Table S2 and S3. To test the difference of the mean values of the BSA between the two chains of TCR or Fab, a t-test was run in which a P-value < 0.05 indicates a significant difference. The P-values obtained for each case are provided in Table S4. T-tests were run in Microsoft Office Excel.

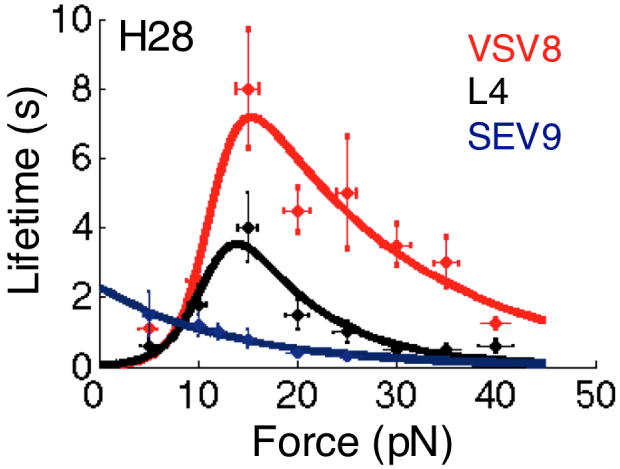

Figure S1: Force-lifetime relationship for wtN15αβ in the presence of H28. Effect of H28 on bond lifetime vs Force diagram shows that bond lifetime increases with force up to 15 pN (Catch bond) followed by decrease of lifetime with force for VSV8 (red) and L4 (black). For sev9 (Blue) the bond lifetime decreases with increment of force. The data are presented along with the fitted curve (solid line).

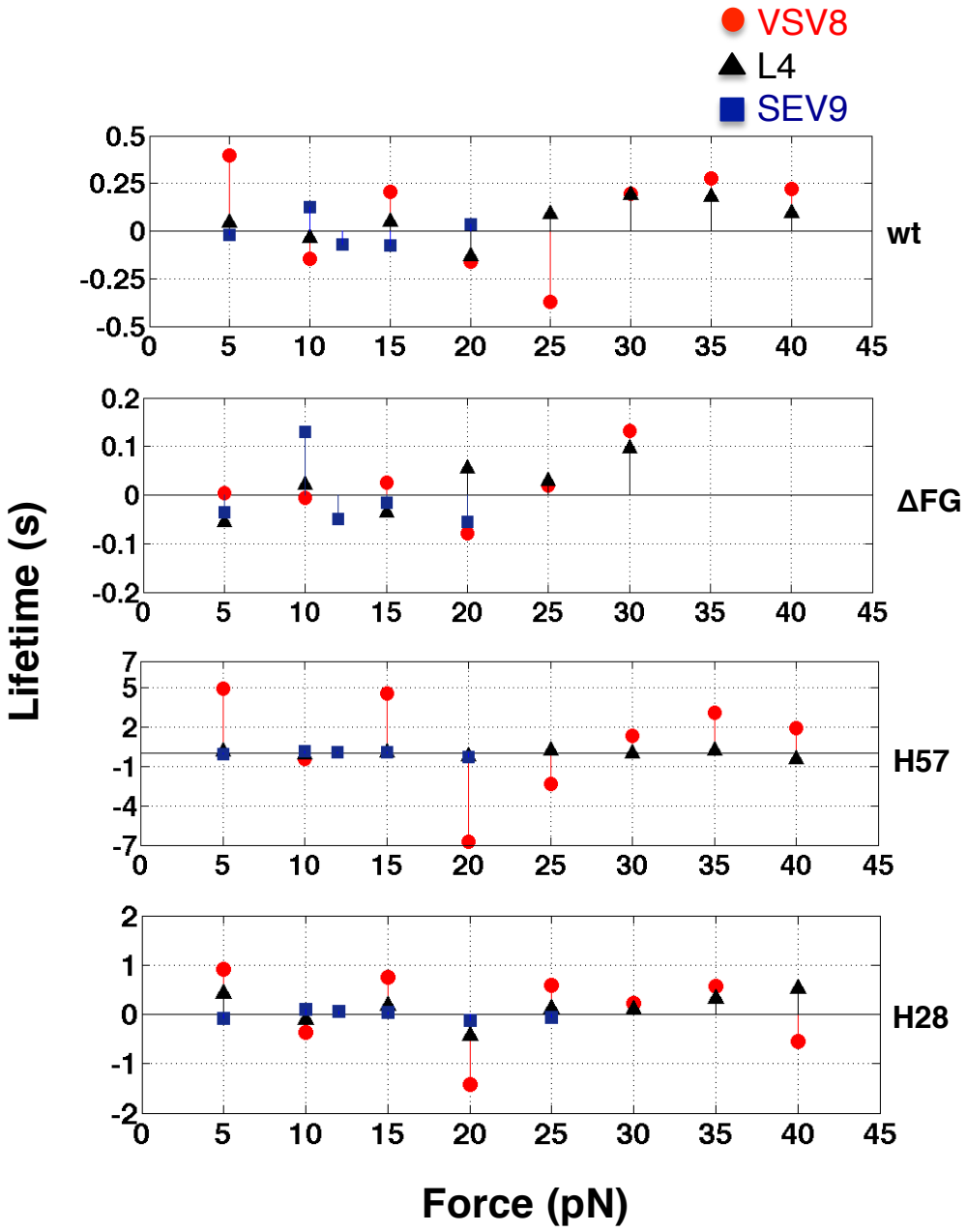

Figure S2: Residual plots from catch bond fits in Figure 2 and Figure S1.

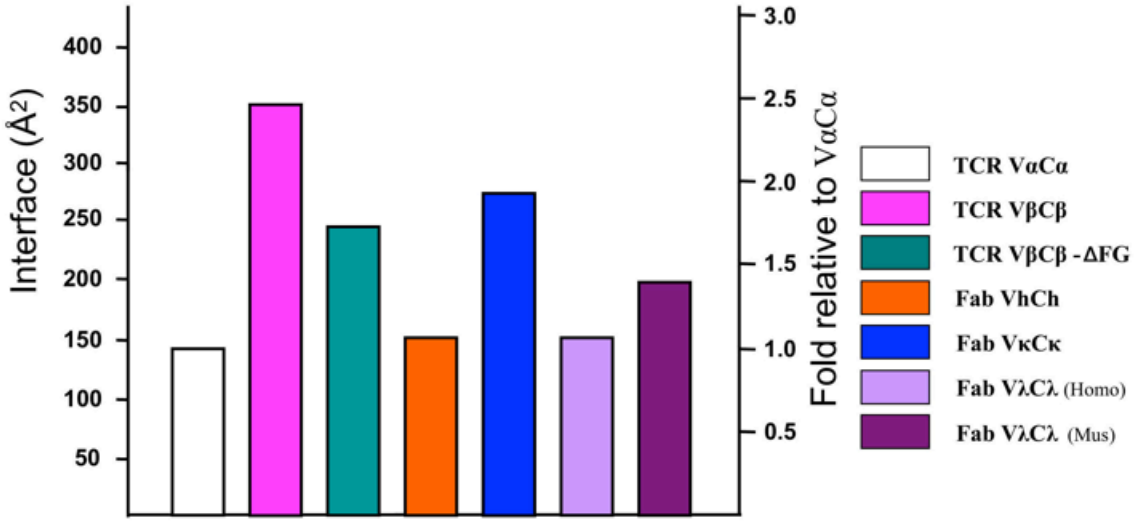

Figure S3: Surface area of each species and its ratio relative to VαCα.

Table S1: Catch Bond fits

	Catch State		Slip State	
Systems	**k_{off}, [s^{-1}]**	**x_β, [nm]**	**k_{off}, [s^{-1}]**	**x_β, [nm]**
wtN15αβ /VSV8[1]	40±102	-1.8±1	0.05±0.03	0.45±0.1
wtN15αβ/L4[2]	9±7	-1.2±0.4	0.01±0.01	1±0.2
wtN15αβ SEV9[3]			0.63±0.1	0.65±0.1
N15αβΔFG/VSV8[4]	9.8±5	-1.4±0.3	0.04±0.03	0.8±0.2
N15αβΔFG/L4[5]	9.8*±9.2	-1.16±0.7	0.02±0.06	1.3±0.8
N15αβΔFG/SEV9[6]			0.92±0.2	0.44±0.1
wtN15αβ /VSV8/FH57[7]	1106±390	-4±1	0.01±0.006	0.25±0.09
wtN15αβ L4/FH57[8]	22±13	-1.9±0.2	0.03±0.003	0.30±0.02
wtN15αβ /SEV9/FH57[9]			0.21±0.02	0.35±0.04
wtN15αβ /VSV8/H28[10]	53.3±11	2.2±0.2	0.05±0.01	0.25±0.05
wtN15αβ /L4/H28[11]	28±4	1.82±0.4	0.04±0.02	0.5±0.1
wtN15αβ /SEV9/H28[12]			0.22±0.05	0.31±0.1

Total number of events: [1] = 314, [2] = 209, [3] = 224, [4] = 294, [5] = 210, [6] = 175, [7] = 178, [8] = 156, [9] = 162, [10] = 264, [11] = 186, [12] = 276

* Constraint limit = 9.8

Table S2. PDB Data analysis for αβTCR in human and mouse.

PDB ID (resolution)	Type/Sapiens	BSA of CαVα ($Å^2$)	BSA of CβVβ(ΔFG) ($Å^2$)	Ratio of β/α (ΔFG)
1LP9 (2.00Å)	Class I αβTCR (*Homo*)	138.3	313.4 (209.5)	2.27 (1.51)
1OGA (1.40Å)	Class I αβTCR (*Homo*)	154.4	408.0 (303.0)	2.64 (1.96)
2BNQ (1.70Å)	Class I αβTCR (*Homo*)	143.2	371.4 (269.1)	2.59 (1.88)
3MV7 (2.00Å)	Class I αβTCR (*Homo*)	144.0	389.3 (250.3)	2.70 (1.74)
3QFJ (2.29Å)	Class I αβTCR (*Homo*)	116.7	297.1 (228.5)	2.55 (1.96)
3VXS (1.80Å)	Class I αβTCR (*Homo*)	145.1	388.8 (284.4)	2.68 (1.96)
4G9F (1.90Å)	Class I αβTCR (*Homo*)	217.9	388.8 (285.1)	1.66 (1.31)
2CKB (3.00Å)	Class I αβTCR (*Mus*)	168.7	295.4 (216.7)	1.75 (1.28)
3RGV (2.90Å)	Class I αβTCR (*Mus*)	153.9	324.7 (231.0)	2.11 (1.50)
3TJH (2.12Å)	Class I αβTCR (Mus)	161.4	320.6 (233.1)	1.99 (1.44)
1FYT (2.60Å)	Class II αβTCR (*Homo*)	121.5	346.9 (230.1)	2.86 (1.89)
1J8H (2.40Å)	Class II αβTCR (*Homo*)	133.3	344.2 (236.4)	2.58 (1.77)
3PL6 (2.55Å)	Class II αβTCR (*Homo*)	95.2	296.1 (199.5)	3.11 (2.10)
4GKZ (2.39Å)	Class II αβTCR (*Homo*)	116.2	354.8 (247.7)	3.05 (2.13)
4MAY (2.20Å)	Class II αβTCR (*Homo*)	94.1	302.2 (201.6)	3.21 (2.14)
3C5Z (2.55Å)	Class II αβTCR (*Mus*)	138.9	366.1 (240.4)	2.64 (1.73)
3QIB (2.70Å)	Class II αβTCR (*Mus*)	127.5	388.9 (244.5)	3.05 (1.92)
3RDT (2.70Å)	Class II αβTCR (*Mus*)	131.0	312.1 (212.4)	2.38 (1.62)
2PYF (2.20Å)	αβTCR (*Homo*)	140.2	313.1 (222.4)	2.23 (1.59)
2XNA (2.10Å)	αβTCR (*Homo*)	95.4	401.4 (298.8)	4.21 (3.13)
3MFF (2.00Å)	αβTCR (*Homo*)	217.7	433.7 (331.0)	1.99 (1.52)
4L4V (1.90Å)	αβTCR (*Homo*)	160.5	433.7 (286.0)	2.42 (1.78)
Average		141.6	354.1 (248.2)	2.50 (1.75)

Table S3. PDB Data analysis for human and mouse antibody Fabs

PDB ID (resolution)	Type/Sapiens	BSA of C*h* and V*h* ($Å^2$)	BSA of Cλ and Vλ ($Å^2$)	BSA of Cκ and Vκ ($Å^2$)	Ratio of h/λ or h/κ
3ZL4 (1.95 Å)	A17 (*Homo*)	184.5	153.7		1.2
4FQ2 (1.90 Å)	10-1074 (*Homo*)	246.9	181.9		1.36
4FZE (2.00 Å)	N26_I1 (*Homo*)	244.4	167.9		1.46
4M5Y (1.55 Å)	5J8 (*Homo*)	166.0	134.7		1.23
4OCR (1.90 Å)	CAP256-VRC26.01 (*Homo*)	201.1	234.8		0.86
4M1D (1.80 Å)	MAB 447-52D (*Homo*)	94.1	168.8		0.56
4LLU (2.16 Å)	PERTUZUMAB (*Homo*)	159.8	131.2		1.22
4EOW (1.97 Å)	MB007 IGG1 (*Homo*)	90.4	103.9		0.87
4FQH (2.05 Å)	CR9114 (*Homo*)	155.3	169.6		0.92
3UJI (1.60 Å)	2558 (*Homo*)	157.8	161.4		0.98
3QHZ (1.55 Å)	2D1 (*Homo*)	211.4	200.6		1.05
Average (Homo)		173.8	164.4		h/λ=1.06
2ZPK (1.80 Å)	P20.1 (*Mus*)	135.6	219.6		0.62 (λ/h=1.62)
2QHR (2.00 Å)	13F6-1-2 (*Mus*)	77.8	125.6		0.62 (λ/h=1.61)
1NGQ (2.40 Å)	1G9 (*Mus*)	172.0	264.8		0.65 (λ/h=1.54)
2OP4 (2.85 Å)	RS2-1G9 *(Mus*)	152.8	215.7		0.71 (λ/h=1.53)
Average (Mus)		134.6	206.4		0.65 (λ/h=1.53)
4G5Z (1.83 Å)	CANAKINUMAB (*Homo*)	130.7		296.4	0.44
4IOI (1.95 Å)	TRASTUZUMAB (*Homo*)	145.4		337.5	0.43
4JDV (1.65 Å)	NIH45-46 ERM-LINE (*Homo*)	141.9		231.8	0.61
4JFX (1.95 Å)	PHOSPHOTYROSINE (*Homo*)	131.3		236.4	0.56

4JHA (1.60 Å)	D25 (*Homo*)	211.4		323.7	0.65
4KQ3 (1.92 Å)	CNTO3186 (*Homo*)	257.2		317.3	0.81
4M7K (1.90 Å)	10H10 (*Homo*)	208.1		318.6	0.65
4NUG (1.86 Å)	PGT151 (*Homo*)	117.2		233.3	0.50
4O4Y (1.85 Å)	2095-2 (*Homo*)	145.2		285.0	0.51
4KMT (2.10 Å)	5-51/O12 (*Homo*)	123.2		193.5	0.64
Average (Homo Fab-κ)		161.2		277.4	0.58 (h/κ) (κ/h=1.72)
4MA7 (1.97 Å)	POM1 (*Mus*)	93.0		249.1	0.37
4OKV (1.80 Å)	8H7 MAB (*Mus*)	223.5		299.0	0.75
1ZEA (1.78 Å)	TE33 (*Mus*)	142.6		334.7	0.43
2AJX (1.85 Å)	7A1 (*Mus*)	165.4		330.1	0.50
3OZ9 (1.60 Å)	NC-1 (*Mus*)	181.4		298.2	0.61
3EYU (2.71 Å)	ROR2 (*Mus*)	119.5		184.3	0.65
Average (Mus Fab-κ)		152.2		286.8	0.55 (h/κ) (κ/h=1.83)
AVERAGE (mus+homo Fab-κ)		158.6		279.3	0.57 (h/κ) (κ/h=1.76)

Table S4. Summary of the statistics

Item / Mean (SD) / Entity	Interface of CαVα or ChVh ($Å^2$)	Interface of CβVβ or CκVκ or CλVλ ($Å^2$)	t-test (P-value)	Ratio CβVβ :CαVα CκVκ :ChVh CλVλ :ChVh	Fold of CβVβ/CκVκ/ CλVλ relative to CαVα
TCRαβ	141.0 (32.4)	354.1 (45.3)	<0.0001	2.50	2.50
TCRαβ (ΔFG)	141.0 (32.4)	248.2 (36.1)	<0.0001	1.75	1.75
Fab-κ	158.6 (45.4)	279.3 (50.7)	<0.0001	1.76	1.98
Fab-λ (Human)	173.8 (51.7)	164.4 (35.2)	0.4926	0.95	1.17
Fab-λ (Mus)	134.6 (40.6)	206.4 (58.3)	0.0059	1.53	1.46

References

1. Touma M*, et al.* (2006) The TCR C beta FG loop regulates alpha beta T cell development. *J Immunol* 176:6812-6623.
2. Keskin DB*, et al.* (2011) Direct identification of an HPV-16 tumor antigen from cervical cancer biopsy specimens. *Frontiers in immunology* 2:75.
3. Bell GI (1978) Models for the specific adhesion of cells to cells. *Science* 200(4342):618-627.
4. Guo B & Guilford WH (2006) Mechanics of actomyosin bonds in different nucleotide states are tuned to muscle contraction. *Proceedings of the National Academy of Sciences of the United States of America* 103(26):9844-9849.
5. Pereverzev YV, Prezhdo OV, Forero M, Sokurenko EV, & Thomas WE (2005) The two-pathway model for the catch-slip transition in biological adhesion. *Biophysical journal* 89(3):1446-1454.